If the Trap Fits: Immunomagnetic Isolation of Spermatozoa

Julia Wang, MS[1*]; Sheree Hughes, PhD[1], Brendan Chapman, BSc[2]; Andrew Currie, PhD[2]; Rachel Houston, PhD[1]

[1]Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340
[2]Medical, Molecular and Forensic Science, Murdoch University, Perth, Australia

ABSTRACT

Sexual assault kit biological evidence is typically composed of majority female epithelial cells with relatively few spermatozoa. The currently accepted cell separation methodology is labor-intensive and time-consuming, which has driven the need for alternative differential extraction methods.

In this study, antibodies for sperm-specific proteins SPAM1, SPACA1, and ZPBP were used to isolate spermatozoa. Although immunocytochemistry confirmed protein expression and antibody-binding capabilities, the results suggest that magnetic beads natively bound both spermatozoa and vaginal epithelial cells without any antibody conjugation. Addition of blocking agent and longer incubation times increased non-specific interaction between cells and beads.

INTRODUCTION

Prevalence of sexual assault and rape in the U.S. is high, with over 400,000 victimizations of rape and sexual assault occurring in 2019[1]. Forensic DNA laboratories frequently process sexual assault kit evidence. Conventional methods of separating female victim epithelial cells from male perpetrator spermatozoa for DNA analysis can be laborious and time-consuming. These workflows utilize preferential lysis extraction where spermatozoa are selectively lysed with reducing agents. Concerns associated with this method include processing time and loss of DNA during extraction[2].

Biomolecule targeting cell isolation methods are widely used in clinical and research settings. The unique function of spermatozoa signify the diverse range of molecules specific to sperm. The proteins targeted in this study were hyaluronidase PH-20 (SPAM1), sperm acrosome membrane-associated protein 1 (SPACA1), and zona pellucida binding protein 1 (ZPBP1). Previous studies show improvement in sperm cell enrichment using these target proteins[3,4]. The objective of this study was to demonstrate that magnetic beads conjugated to antibodies for these targets can isolate sperm cells from mixtures.

RESULTS AND DISCUSSION

Antibody Binding Confirmation

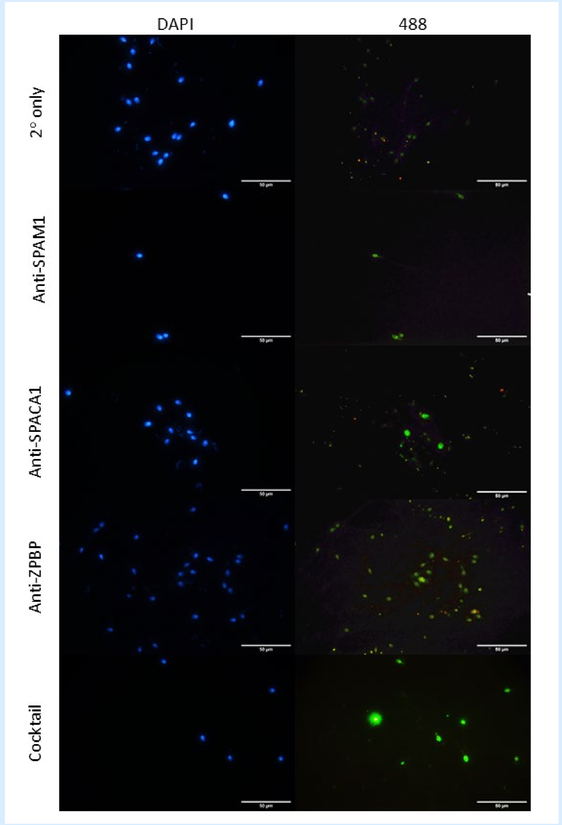

Figure 1. Target protein binding confirmation by immunocytochemistry in spermatozoa. Each target protein was detected by anti-SPAM1, anti-SPACA1, anti-ZPBP, or combined in an antibody cocktail. Scale bars represents 50 µm.

Antibody Cocktail-Bead Specificity

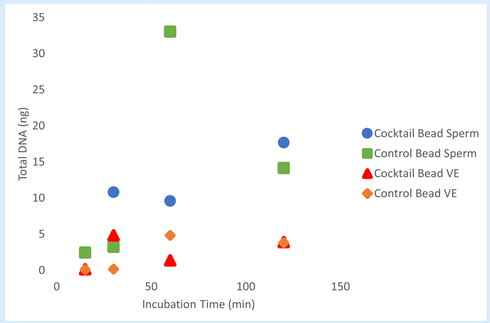

Figure 2. Non-specific interaction between BSA-blocked magnetic beads and cells. Increasing incubation time increased non-specific interactions between the magnetic bead complexes and both spermatozoa and vaginal epithelial (VE) cells.

Antibody Cocktail-Bead Sensitivity

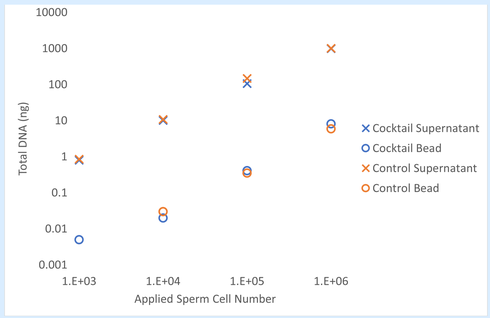

Figure 3. Minimal differences observed between control beads and antibody cocktail-conjugated beads. Majority of cells were found in supernatant fraction rather than the bead-complex fraction.

- Each antibody successfully confirmed expression of the respective target proteins in single antibody staining as well as in a combined antibody cocktail (**Figure 1**).
- While limited relative to spermatozoa, vaginal epithelial cells were capable of binding to the magnetic bead even in the presence of blocking agents (**Figure 2**).
- Lack of specificity and sensitivity for spermatozoa capture (**Figure 3**) suggests that the antibody-bead complexes require refinement such as in antibody conformation or bead-antibody conjugation chemistry.

MATERIALS AND METHODS

Immunocytochemistry

- 4% Formaldehyde fixation, R.T. 15 minutes
- 5% Normal goat serum blocking, R.T. 2 hours
- 1° Antibody incubation, 4°C overnight
- 2° Antibody & DAPI incubation, R.T. 1 hour
- Leica FS CB microscope

Bead Preparation and Cell Isolation

- Performed following manufacturer recommendations with Dynabeads® M-450 Epoxy (Thermo Fisher Scientific, Waltham, MA).
- Antibodies used were anti-rabbit IgG polyclonal anti-SPAM1, anti-SPACA1, and anti ZPBP (Thermo Fisher Scientific)

DNA Extraction, Quantification, and Isolation

Bead-isolated sperm or vaginal epithelial cells
↓
QIAamp® DNA Investigator Kit
↓
Investigator® Quantiplex® Pro Kit
↓
Investigator® 24plex QS kit
↓
3500 Genetic Analyzer
↓
GeneMapper® ID-X v1.4.

CONCLUSIONS

- Antigen-binding capacity of all three antibodies were confirmed by immunocytochemistry.
- Lack of difference between beads with and without antibodies demonstrated non-specific interaction between cells and beads.
- Vaginal epithelial cell cross-reactivity with the magnetic beads was present.

REFERENCES

1. Morgan RE, Truman JL. Criminal Victimization, 2019. Bureau of Justice Statistics, 2020.
2. Vuichard S, Borer U, Bottinelli M, Cossu C, Malik N, Meier V, et al. Differential DNA extraction of challenging simulated sexual-assault samples: a Swiss collaborative study. Investig Genet 2011;2:11. https://doi.org/10.1186/2041-2223-2-11.
3. Jackson MB. Developing methods for antibody-mediated enrichment and capture of spermatozoa from complex cell mixtures for forensics applications. 2019.
4. Zhao X-C, Wang L, Sun J, Jiang B-W, Zhang E-L, Ye J. Isolating Sperm from Cell Mixtures Using Magnetic Beads Coupled with an Anti-PH-20 Antibody for Forensic DNA Analysis. PLOS ONE 2016;11(7):e0159401. https://doi.org/10.1371/journal.pone.0159401.

ACKNOWLEDGEMENTS

Samples were collected from donors using SHSU IRB protocol 2020-248. This work was supported by financial support of the Forensic Sciences Foundation Lucas Grant. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Forensic Sciences Foundation.